EMBO reports

scientific report

Messenger RNA conformations in the ribosomal E site revealed by X-ray crystallography

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A comparison of messenger RNA in X-ray crystal structures of 70S ribosomal complexes in the initiation, post-initiation and elongation states of translation shows distinct conformational differences in the exit (E) codon. Here, we present structural evidence indicating that, after the initiation event, the E codon nucleotides relax and form a classical A-helical conformation. This conformation is similar to that of the P and A codons, and is favourable for establishing Watson–Crick interactions with the anticodon of E-site transfer RNA.

Keywords: ribosome; tRNA; mRNA; structure; E-codon
EMBO reports (2007) **8**, 846–850. doi:10.1038/sj.embor.7401044

INTRODUCTION

All cell proteins are synthesized by the ribosome, the cellular machinery on which amino acids are incorporated into the nascent polypeptide chain according to the sequence specified by messenger RNA. During the translational process, transfer RNAs move through three tRNA-binding sites on the ribosome: the aminoacyl-tRNA-binding site (A site), the peptidyl-tRNA-binding site (P site) and finally the exit site (E site), from where the deacylated tRNA leaves the ribosome. The E site was initially proposed by Rheinberger *et al* (1981), and it has been shown that newly deacylated tRNA from the P site is translocated to the E site after formation of the peptide bond (Kirillov *et al*, 1983; Lill *et al*, 1984). The features and functions of the E site have been examined in a large number of crosslinking, chemical footprinting, protection and kinetic studies (Lill & Wintermeyer, 1987; Moazed & Noller, 1989; Wower *et al*, 1993; Rinke-Appel *et al*, 1995; Joseph & Noller, 1996; Bocchetta *et al*, 2001); however, the function of the ribosomal E site remains ambiguous. One of the widely discussed issues is whether cognate tRNA in the E site will maintain the codon–anticodon interaction with the E codon of mRNA, which could be a prerequisite for achieving high-precision decoding and reading-frame maintenance through a reciprocal 'A-site/E-site' mechanism (Geigenmuller & Nierhaus, 1990; Márquez *et al*, 2004), or whether the role of the E site is to facilitate the release of deacylated tRNA during translocation (Lill & Wintermeyer, 1987). The three ribosomal tRNA-binding sites were initially visualized by low-resolution cryo-electron microscopic (cryo-EM) studies, which confirmed that all three sites are located on the interface between the 30S and 50S subunits (Agrawal *et al*, 1996; Stark *et al*, 1997). The interaction of mRNA with full-length tRNA molecules in the three sites was later described at higher resolution when the 5.5 Å X-ray structure of the 70S ribosome in the initiation state was determined (Yusupov *et al*, 2001). The conformation of the E codon of mRNA and the anticodon stem–loop of E-site tRNA in this complex does not allow the formation of a codon–anticodon interaction. Recently, the crystal structure of the 70S ribosome in complex with mRNA containing a Shine–Dalgarno sequence—$tRNA^{fMet}$ in the P site, and $tRNA^{Phe}$ in the A and E sites—was determined (Selmer *et al*, 2006). The E codon was found in a conformation comparable to the E codon in the initiation complex (Yusupov *et al*, 2001). Another X-ray structure of a 70S ribosomal complex in the post-translocational elongation state was recently solved (Korostelev *et al*, 2006); however, the E codon and anticodon stem–loop of the E-site tRNA were largely disordered, so no reliable interpretation could be made.

Here, we report on the comparison of conformations of the E codon of mRNA and the anticodon of E site of tRNA in crystal structures of 70S ribosomal complexes in different functional states corresponding to initiation, post-initiation and elongation states of translation.

RESULTS

The ribosomal complexes

Structural determination was carried out by using X-ray analysis on crystals of *Thermus thermophilus* 70S ribosome functional complexes diffracting to a maximum resolution of 3.9 Å (Yusupova *et al*, 2006). The initiation complex consisted of 70S ribosome, $tRNA^{fMet}$ in the P site and $GGC(SD)(A)_4AUG(A)_9$ mRNA, in which SD is the Shine–Dalgarno (AAGGAGGU) sequence that is complementary to the 3′-terminal sequence of 16S ribosomal RNA (Fig 1A). The post-initiation complex was formed from 70S ribosome, elongator $tRNA^{Phe}$ in the A and P sites, and $GGC(SD)(U)_9UUU(U)_4$ mRNA (Fig 1B). This mRNA was designed without the initiation AUG codon to determine the reading frame; therefore, the ribosome had a 'free choice' of start codon.

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Received 2 May 2007; revised 27 June 2007; accepted 3 July 2007; published online 3 August 2007

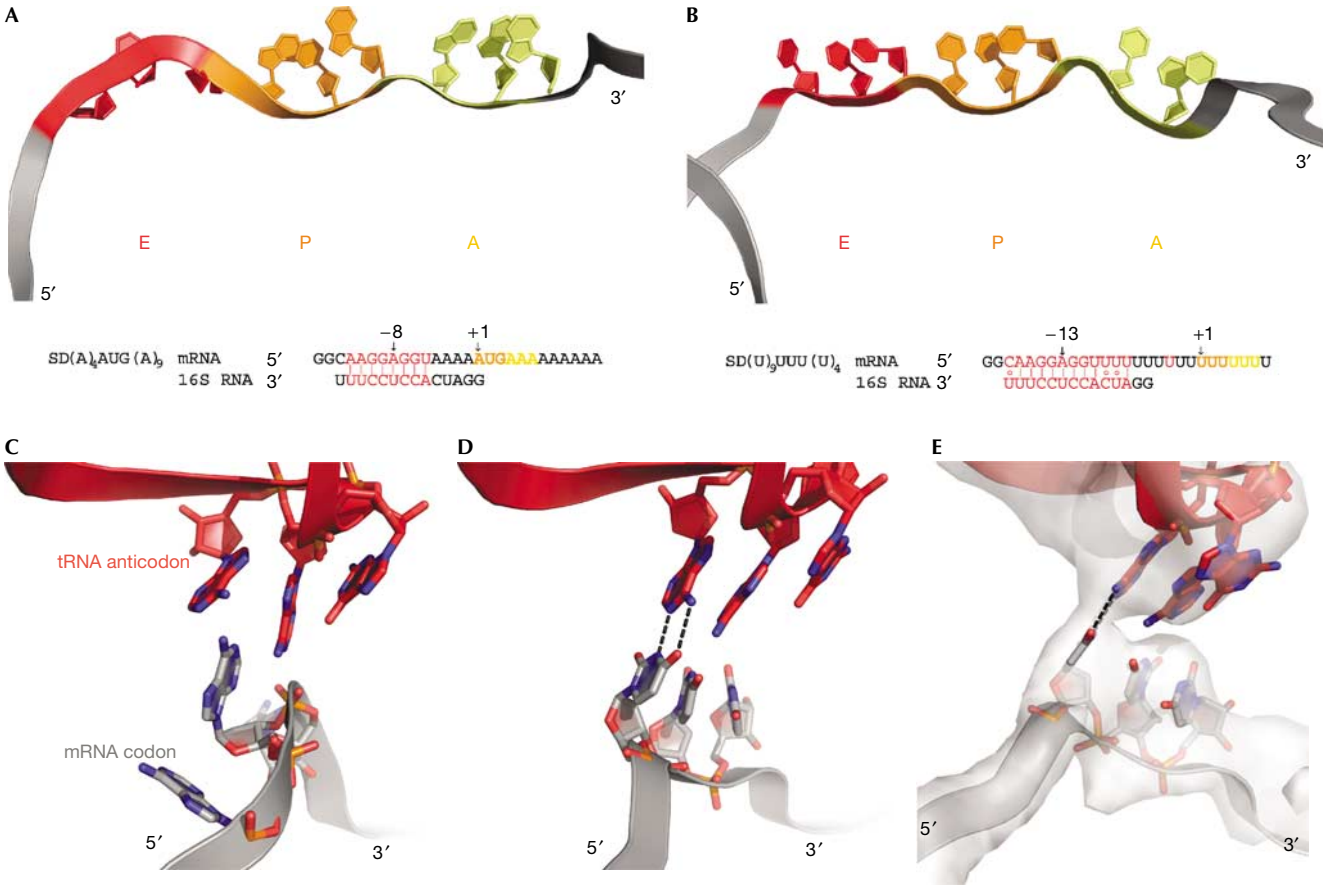

Fig 1 | Codon–anticodon interaction in the E site. (**A**,**B**) Structure of E, P and A codons in initiation (**A**) and post-initiation complexes (**B**). Sequences of the messenger RNA (mRNA) and the Shine–Dalgarno (SD) duplex (in red) are also shown. (**C**,**D**) E-site tRNA interaction with the E anticodon in initiation (**C**) and post-initiation (**D**) complexes. The E codon can adopt the classical A-helical conformation that enables base pairing with the anticodon of E-site tRNA only in the post-initiation complex. (**E**) Fourier (F_o-F_c) electron density map calculated at a resolution of 5.5 Å with E-site tRNA and mRNA omitted from the model. E site, exit site.

This complex can be considered to be a good model for the post-initiation ribosomal state when the Shine–Dalgarno interaction between mRNA and 16S RNA still exists, while the elongator $tRNA^{Phe}$ are already bound to the P and A codons. The ribosomal complex representing the elongation state was formed using $(U)_{12}AUG(U)_9$ mRNA without a Shine–Dalgarno sequence; the AUG codon was added to the polyU mRNA to increase the efficiency of complex formation. All the 70S ribosomal complexes described here contained E-site tRNA, which was co-purified with the ribosome sample in stoichiometric amounts and was a mixture of all tRNAs (G. Keith & M. Yusupov, unpublished data).

Structure of the E codon

On the basis of a comparison of the crystal structures of the post-initiation and initiation ribosomal complexes, it is believed that after the initiation of translation, the mRNA must move in the 3′–5′ direction with a simultaneous clockwise rotation and lengthening of the Shine–Dalgarno duplex. In addition, there is an increase from four to six nucleotides in the distance between the 3′-end of the Shine–Dalgarno helix and the P codon, indicating a conformational relaxation (Yusupova *et al*, 2006). Both (F_o-F_o) difference Fourier maps and (F_o-F_c) omit maps—with mRNA and E tRNA omitted from the model—for the post-initiation complex calculated at a resolution of 5.5 Å show density for the mRNA E codon (position −3 to −1) and the E-site tRNA anticodon stem–loop. This is of sufficient quality to allow a confident construction of the model, with only one possible conformation of the main chain of mRNA (Fig 1E). An interesting feature of the post-initiation complex is that the mRNA forms an E codon with the classical A-helical shape, similar to that of the P and A codons, which is a prerequisite for cognate codon–anticodon interactions with the tRNA. This differs from the crystal structure of the initiation complex, in which the E codon of the mRNA has a conformation that is distinctly different from the classical A-helical shape. Fig 1A,C shows that in the initiation complex the first and third nucleotides of the mRNA E codon are flipped out, so that the codon conformation does not allow the formation of base pairs with the tRNA anticodon. In the more relaxed mRNA of the post-initiation complex, all three nucleotides (−3 to −1) are in the conventional A-helical conformation (Fig 1B). The E codon nucleotides are found in an orientation favourable for base pairing with the anticodon of E tRNA, although the 'wobble' nucleotide of

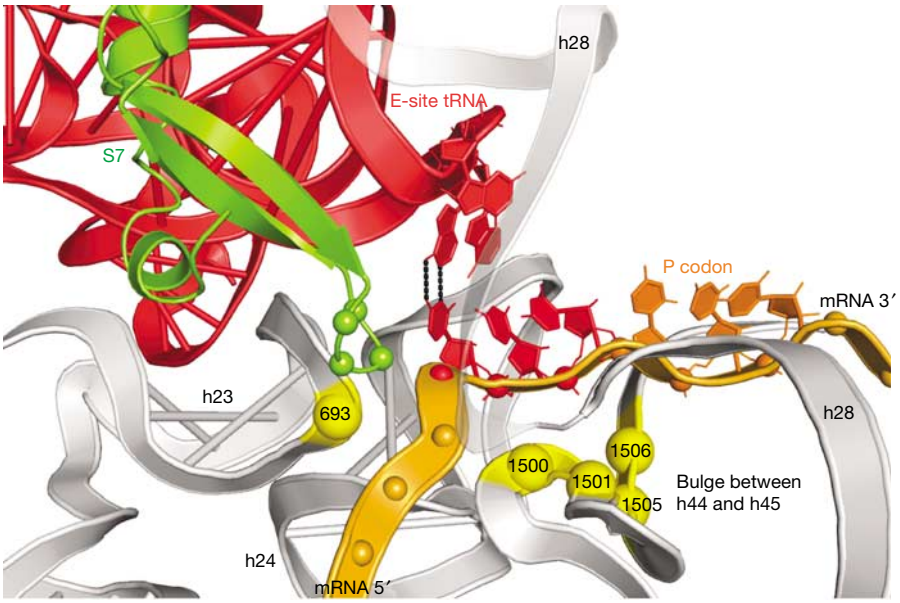

Fig 2 | A detailed view of the ribosomal environment of the E codon in the post-initiation complex. Nucleotides directly involved in the stabilization of the E codon triplet structure are shown as yellow spheres. The residues of the S7 β-hairpin (shown as green spheres) are not in direct contact with the E codon, but are instead interacting with the upstream bases. E, exit site; h, helix; mRNA, messenger RNA; tRNA, transfer RNA.

the E-site tRNA anticodon is slightly distorted (Fig 1D). Furthermore, we observe continuous electron density between at least the first nucleotide (position −3) of the E codon and nucleotide 34 of the E-site tRNA anticodon (Fig 1E). In both initiation and post-initiation complexes, the E codon of mRNA is positioned on top of the bulge between helix 44 and helix 45 of the 16S ribosomal RNA with specific interactions with nucleotides 1499, 1500, 1505 and 1506, and is also in direct contact with nucleotide 693 from helix 23 (Fig 2). The nucleotides 788–790 from helix 24 are also in the immediate vicinity of the E codon, although no direct contacts are seen. Contrary to what was reported for the initiation complex (Yusupova *et al*, 2001), the E codon of the mRNA in the post-initiation complex is no longer interacting with the β-hairpin of protein S7. Owing to relaxation of the mRNA and formation of the classical codon triplet, this hairpin is now close to positions −4 and −5 of the post-initiation mRNA (Fig 2).

In the crystal structure of the ribosomal complex representing the elongation step, in which the mRNA does not contain a Shine–Dalgarno sequence, the E codon conformation appears to be identical to that in the post-initiation complex (data not shown).

DISCUSSION

In recent years, the determination of several new crystal structures of 70S ribosomal complexes containing tRNAs and mRNA, representing various stages of the translation cycle, has provided a greatly enhanced view of the details of ribosome–mRNA–tRNA interactions (Yusupova *et al*, 2001; Berk *et al*, 2005; Jenner *et al*, 2005; Petry *et al*, 2005; Korostelev *et al*, 2006; Selmer *et al*, 2006). However, it is clear that many aspects are far from completely understood, such as the role of the ribosomal E site.

The 5.5 Å X-ray structure of a 70S ribosome initiation complex showed that the mRNA E codon is in a conformation that does not allow the formation of base pairs with the tRNA anticodon (Yusupov *et al*, 2001). Two groups have independently reported crystal structures of the 70S *T. thermophilus* ribosome in complex with tRNAs and mRNA (Korostelev *et al*, 2006; Selmer *et al*, 2006). Selmer and co-workers determined the 2.8 Å crystal structure of a pre-translocation complex containing 70S ribosome, $tRNA^{fMet}$ in the P site, $tRNA^{Phe}$ in A and E sites, and an mRNA containing the Shine–Dalgarno sequence and therefore in a state that closely resembles the initiation complex described by Yusupov *et al* (2001). Although the Shine–Dalgarno duplex was disordered, the E codon and two additional downstream nucleotides were ordered, and the conformations of the E a codon and the anticodon stem–loop of the E-site tRNA clearly show that no codon–anticodon interaction is possible in this complex (Selmer *et al*, 2006). This is in agreement with our data from the crystal structure of the initiation complex, in which the E codon is in a comparable conformation excluding the possibility of forming a codon–anticodon interaction (Fig 1C). This is to be expected as the genuine initiation complex does not contain deacylated tRNA in the E site. Korostelev *et al* (2006) presented the 3.7 Å crystal structure of a 70S ribosomal complex in the post-translocational elongation state containing $tRNA^{Phe}$ in the P site, co-purified tRNAs in the E site and a decamer mRNA. In this structure, the E codon and anticodon stem–loop of the E tRNA were not well resolved, so no conclusion could be drawn about the codon–anticodon interaction in the E site.

We have shown that during initiation, the mRNA extending from the P codon to the first base pair of the Shine–Dalgarno duplex is stretched out, so that the E codon nucleotides are distorted into a conformation that is unable to form codon–anticodon interactions. However, after the initiation of translation, while the Shine–Dalgarno interaction between the mRNA and 16S RNA still exists and the elongator tRNAs are already bound to the P and A codons, the Shine–Dalgarno duplex undergoes a

positional shift. The tension in the mRNA seems to be released, causing the E codon to adopt the classical A-helical conformation, similar to that of the P and A codons. This gives rise to the possibility of forming codon–anticodon interactions in the E site. The electron density further suggests (Fig 1E) that although the tRNA in the E site is a mixture of all tRNAs, co-purified in stoichiometric ratios with the ribosome sample, there is indeed some base pairing, at least between the first nucleotide (position −3) of the E codon and the third nucleotide of the E-site tRNA anticodon.

These data seem to support speculations based on observations obtained from cryo-EM reconstructions of ribosomal complexes (Agrawal *et al*, 2000), that during translation the tRNAs move from the A site on the ribosome to the P site and then from the P site to the E site together with the mRNA while keeping the codon–anticodon interactions intact. It was proposed that the next step entails the release of the codon–anticodon interaction in the E site and a shift of the E-site tRNA further away from the E site, which results in dissociation of the tRNA from the ribosome. A cryo-EM study visualized two E-site tRNA-binding sites, named E and E2 (Agrawal *et al*, 2000), which seem to be related to the E′ and E sites, respectively, as described by Robertson *et al* (1986). According to Robertson *et al* (1986), the E′ site is a codon–anticodon interaction-dependent and short-lived tRNA position, from where the tRNA moves to the E site, which has no codon–anticodon interaction, and from where the tRNA dissociates from the ribosome. Agrawal and co-workers identified their E site as Robertson's E′ site, with all the E′-site features except that they observed a relatively stable binding of tRNA. On the basis of the close proximity of the anticodon stem–loop of the tRNA in E site to that of P-site tRNA, Agrawal *et al* (2000) suggested that the codon–anticodon interaction is feasible at this stage. A weakly occupied E2 site (E site according to Robertson) was suggested as being a short-lived tRNA-binding position (Agrawal *et al*, 2000), which might be a further site for deacylated tRNA on the way out of the ribosome.

The recently published X-ray structure of the ribosomal pre-translocation complex (Selmer *et al*, 2006) showed stable binding of tRNA to the ribosomal E site in a codon–anticodon interaction-independent manner in a position that differs markedly—especially in the anticodon stem–loop part—from the E-site tRNA described in the present study. These data indicate the existence of different intermediate positions for deacylated tRNA in the ribosomal E site, before its release from the ribosome.

The X-ray structures of the ribosome post-initiation and elongation complexes described here might represent the state of translation when the tRNA is bound to the E site and base pairing is maintained between the E codon and anticodon of the tRNA. The next step involves dissociation of the codon–anticodon interaction and possible displacement of the tRNA to an intermediate E-site position. Subsequently, the deacylated tRNA is released from the ribosome.

METHODS

Ribosome preparation, complex formation and crystallization. 70S ribosomes were isolated from *T. thermophilus* HB8 cells grown by large-scale fermentation, as described by Gogia *et al* (1986). The 70S ribosomal complexes were prepared and crystallized as described by Yusupova *et al* (2006). The ligands, synthetic mRNA and tRNA, were present in 1.3- to 2-fold stoichiometric excess of the concentration of ribosomes.

mRNAs and tRNAs. In this study, the following mRNAs were used: (i) GGC(SD)$(A)_4$AUG$(A)_9$ for the initiation complex; (ii) GGC(SD)$(U)_9$UUU$(U)_4$ for the post-initiation complex; and (iii) $(U)_{12}$AUG$(U)_9$ for the elongation complex. The mRNA samples were created using the solid-phase system (Dharmacon Inc, Boulder, CO, USA) and gel-purified before use in crystallization. $tRNA^{fMet}$ and $tRNA^{phe}$ were isolated from *Escherichia coli* (Chemical Block, Moscow, Russia).

Data collection and processing. All data were collected at cryogenic temperatures at the Synchrotron Light Source (Switzerland), with a typical exposure time of 10 s for an oscillation range of 0.25°. The data were integrated, processed and scaled using the HKL-2000 package (Otwinowski & Minor, 1997).

Model building and refinement. Electron density maps (weighted $2F_o-F_c$) and omit maps (F_o-F_c) were used for model building. By using the no-template ribosomal complex as a reference, difference Fourier maps (F_o-F_o) were used to build models of the mRNA/tRNA ligands and the rRNA conformational changes. The CNS package (Brünger *et al*, 1998) was used for refinement of the original 70S *T. thermophilus* model (Protein Data Bank code 1YL3/1YL4), modified as described previously (Yusupova *et al*, 2006) with further bulk solvent corrections carried out as described by Rees *et al* (2005). Model building and alterations were carried out using the program O (Jones & Kjeldgaard, 1997) and figures were produced with the program PyMOL (DeLano, 2002).

Database accession codes. Coordinates and structural factors were deposited in the Protein Data Bank with accession numbers 2HGR and 2HGU (initiation complex 30S and 50S subunits), and 2HGP and 2HGQ (post-initiation complex).

ACKNOWLEDGEMENTS

We thank M. Rodnina for critical comments and suggestions. This work was supported by institutional funds from Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale, Université Louis Pasteur and by contract number LSHG-CT-2006-031220 from the European Commission as SPINE2-Complexes.

REFERENCES

Agrawal RK, Penczek P, Grassucci RA, Li Y, Leith A, Nierhaus KH, Frank J (1996) Direct visualization of A-, P-, and E-site transfer RNAs in the *Escherichia coli* ribosome. *Science* **271:** 1000–1002

Agrawal RK, Spahn MTC, Penczek P, Grassucci RA, Nierhaus KU, Franck J (2000) Visualization of tRNA movements on the *Escherichia coli* 70S ribosome during the elongation cycle. *J Cell Biol* **150:** 447–459

Berk V, Zhang W, Pai RD, Cate JH (2005) Structural basis for mRNA and tRNA positioning on the ribosome. *Proc Natl Acad Sci USA* **103:** 15830–15834

Bocchetta M, Xiong L, Shah S, Mankin AS (2001) Interactions between 23S rRNA and tRNA in the ribosomal E site. *RNA* **7:** 54–63

Brünger AT *et al* (1998) Crystallography & NMR system: a new software suite for macromolecular structure determination. *Acta Crystallogr D* **54:** 905–921

DeLano WL (2002) *The PyMOL Molecular Graphics System*. San Carlos, CA, USA: DeLano Scientific

Geigenmuller U, Nierhaus KH (1990) Significance of the third tRNA binding site, the E site, on *E. coli* ribosomes for the accuracy of translation: an occupied E site prevents the binding of non-cognate aminoacyl-tRNA to the A site. *EMBO J* **9:** 4527–4533

Gogia ZV, Yusupov MM, Spirina TN (1986) Structure of *Thermus thermophilus* ribosomes. Method of isolation and purification of ribosomes. *Molekul Biol* **20:** 519–526

Jenner L, Romby P, Rees B, Schulze-Brieese C, Springer M, Ehresmann C, Ehresmann B, Moras D, Yusupova G, Yusupov M (2005) Translational operator of mRNA on the ribosome: how repressor proteins exclude ribosome binding. *Science* **308:** 120–123

Jones TA, Kjeldgaard M (1997) Electron-density map interpretation. *Methods Enzymol* **277B:** 173–207

Joseph S, Noller HF (1996) Mapping the rRNA neighborhood of the acceptor end of tRNA in the ribosome. *EMBO J* **15:** 910–916

Kirillov SV, Makarov EM, Semenkov YuP (1983) Quantitative study of interaction of deacylated tRNA with *Escherichia coli* ribosomes. Role of 50S subunits in formation of the E site. *FEBS Lett* **157:** 91–94

Korostelev A, Trakhanov S, Laurberg M, Noller HF (2006) Crystal structure of a 70S ribosome-tRNA complex reveals functional interactions and rearrangements. *Cell* **126:** 1065–1077

Lill R, Wintermeyer W (1987) Destabilization of codon–anticodon interaction in the ribosomal exit site. *J Mol Biol* **196:** 137–148

Lill R, Robertson JM, Wintermeyer W (1984) tRNA binding sites of ribosomes from *Escherichia coli*. *Biochemistry* **23:** 6710–6717

Márquez V, Wilson DN, Tate WP, Triana-Alonso F, Nierhaus KH (2004) Maintaining the ribosomal reading frame: the influence of the E site during translational regulation of release factor 2. *Cell* **118:** 45–55

Moazed D, Noller HF (1989) Interaction of tRNA with 23S rRNA in the ribosomal A, P, and E sites. *Cell* **57:** 585–597

Otwinowski Z, Minor W (1997) Processing of X-ray diffraction data collected in oscillation mode. In *Methods in Enzymology Macromolecular Crystallography*, Carter CW Jr, Sweet RM (eds), Vol 276, part A, pp 307–326. New York, USA: Academic

Petry S, Brodersen D, Murphy FV IV, Dunham CM, Selmer M, Tarry MJ, Kelley AN, Ramakrishnan V (2005) Crystal structures of the ribosome in complex with release factors RF1 and RF2 bound to a cognate stop codon. *Cell* **123:** 1255–1266

Rees B, Jenner L, Yusupov M (2005) Bulk-solvent correction in large macromolecular structures. *Acta Crystallogr D* **61:** 1299–1301

Rheinberger HJ, Sternbach H, Nierhaus KH (1981) Three tRNA binding sites on *Escherichia coli* ribosomes. *Proc Natl Acad Sci USA* **78:** 5310–5314

Rinke-Appel J, Junke N, Osswald M, Brimacombe R (1995) The ribosomal environment of tRNA: crosslinks to rRNA from positions 8 and 20:1 in the central fold of tRNA located at the A, P, and E site. *RNA* **1:** 1018–1028

Robertson JM, Paulsen P, Wintermeyer W (1986) Pre-steady state kinetics of ribosomal translocation. *J Mol Biol* **192:** 351–360

Selmer M, Dunham CM, Murphy FV IV, Weixlbaumer A, Petry S, Kelley AC, Weir JR, Ramakrishnan V (2006) Structure of the 70S ribosome complexed with mRNA and tRNA. *Science* **313:** 1935–1942

Stark H, Orlova EV, Rinke-Appel J, Junke N, Mueller F, Rodnina M, Wintermeyer W, Brimacombe R, van Heel M (1997) Arrangement of tRNAs in pre- and posttranslocational ribosomes revealed by electron cryomicroscopy. *Cell* **88:** 19–28

Wower J, Scheffer P, Sylvers LA, Wintermeyer W, Zimmermann RA (1993) Topography of the E site on the *Escherichia coli* ribosome. *EMBO J* **12:** 617–623

Yusupov MM, Yusupova GZ, Baucom A, Lieberman K, Earnest TN, Cate JH, Noller HF (2001) Crystal structure of the ribosome at 5.5 Å resolution. *Science* **292:** 883–896

Yusupova G, Jenner L, Rees B, Moras D, Yusupov M (2006) Structural basis for messenger RNA movement on the ribosome. *Nature* **444:** 391–394

Yusupova GZ, Yusupov MM, Cate JH, Noller HF (2001) The path of messenger RNA through the ribosome. *Cell* **106:** 233–241